



Highlights:

- Ready-coated plate
- Two hour protocol
- Consistent quality
- Higher throughput

Contents of Kit:

- Anti-Phytophthora antibody-coated strip plate (12 strips of 8 wells each, in frame)
- Phytophthora Enzyme Conjugate
- 1X Leaf Extraction Buffer
- Packet Wash Buffer Salts (makes 1L)
- Substrate
- Stop Solution

Materials Needed:

- Pipette that will measure 100 μ L (multi-channel for large runs)
- racked dilution tubes for loading samples into the plate with a multi-channel pipette (optional)
- marking pen (indelible)
- tape or Parafilm®
- timer
- distilled or deionized water for preparing Wash Buffer
- wash bottle, or microtiter plate or strip washer
- leaf extraction equipment
- centrifuge (optional)
- platform orbital plate shaker with orbital diameter of ≥ 18 mm, set to 150-200 rpm (do not use "microplate shakers" [< 5 mm])
- microtiter plate reader or strip reader capable of reading 450 nanometers (nm)

Catalog Number AP 058

Intended Use

The QualiPlate Kit for *Phytophthora* spp. screens for the presence of several *Phytophthora* species in leaf extracts obtained by different grinding methods. The antibody used in the kit has been shown to be reactive to at least 24 species of *Phytophthora*. The kit has been able to consistently detect the presence of *Phytophthora* species in leaf tissue determined to be *Phytophthora*-positive by other methods.

Preparation of Solutions

- **Wash Buffer:** Add the contents of the packet of Wash Buffer Salts (phosphate buffered saline, pH 7.4 – 0.05% Tween 20) to 1 liter of distilled or deionized water, and stir to dissolve. Store refrigerated when not in use; warm to room temperature prior to assay. Additional 1L dry packets may be purchased from Sigma Chemicals, Cat#P-3563, or similar formulae may be prepared from salts on site.

Sample Preparation

The leaf sample must be extracted with at least 10 volumes (mL/gram) of 1X Leaf Extraction Buffer. For example:

- 0.1 g of leaf tissue : 1 mL of Extraction Buffer

Grind tissue before addition of Buffer. Tissue must be thoroughly macerated. The use of metal, porcelain or glass beads with mechanical device may be necessary to thoroughly macerate waxy leaf tissue samples.

Transfer into suitable container, then add Extraction Buffer. Vortex sample. Note: extracts will be foamy.

Pull off particle-free extract to run in the test. Clarification of extracts by centrifugation is recommended (10 minutes at 1800-5000 x g), but not required.

How to Run the Assay

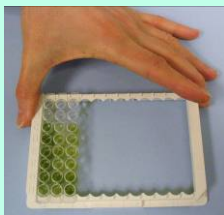
- Read all of these instructions before running the kit.
- Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed plates and reagents at room temperature - do not remove plates from bag with desiccant until they have warmed up).
- **Organize all reagents, sample extracts, and pipettes so that step 1 can be performed in 15 minutes or less;** the use of a multi-channel pipette is strongly recommended for all reagent and extract transfers.
- If more than four strips are to be run at one time, the 15 minutes is likely to be exceeded, and the use of a multi-channel pipette is recommended (see "Note" below).
- If four or fewer strips are to be run, use a disposable-tip air-displacement pipette and a clean pipette tip to add Extraction Buffer or sample extract to the wells. Conjugate, Substrate, and Stop Solution may be added in the same manner; alternatively, use a repeating pipette with a disposable tip on the end of the Combipip for each of the three reagents.



Prepare wash buffer and extraction solutions



Remove unneeded strips



Add Extraction Buffer and sample extracts; mix; incubate



All incubation steps must be performed on an orbital shaker with 18+ mm orbital diameter



Bottle Wash method

- If fewer than all twelve strips are used, reseal the unneeded strips and the desiccant in the foil bag provided and refrigerate.
- Use the well identification markings on the plate edge as a guide when adding the samples and reagents. It is recommended that at least two wells each of Extraction Buffer and known-negative leaf extract be run on each plate. Additional quality control samples may be added at the discretion of the user. Sample extracts may be run in either single or duplicate wells.

1. Add **100 μ L** of **Extraction Buffer**, **100 μ L** of any **user-prepared negative control leaf extract**, and **100 μ L** of each **sample extract** to their respective wells. Follow the same order of addition for all reagents. Treat each plate as an independently timed assay.

NOTE: It is strongly recommended that a multi-channel pipette be used in steps 1, 5, 8 and 9.

2. Thoroughly mix the contents of the wells by moving the plate in a rapid circular motion on the bench top for a full 20-30 seconds. Be careful not to spill the contents!
3. Cover the wells with tape or Parafilm to prevent evaporation and incubate for **30 minutes** at **ambient temperature** on an **orbital shaker (with 18+ mm orbital diameter)** at **150 to 200 rpm**. Note: Shaking during incubation steps is mandatory where called for. Failure to do so will result in up to 50% loss in assay sensitivity.

Protocol option: For testing convenience, at this point samples may be incubated overnight in the refrigerator (up to 16 hours at 5°C). Allow plates to come to room temperature with the rest of the kit reagents the next morning, before going on to step 4.

4. After incubation, carefully remove the covering and empty contents of the wells into a sink or other suitable container by inverting quickly and vigorously shaking the plate. Flood the wells completely with **Wash Buffer**, then empty as directed above. Repeat this wash step at least three times. After the final wash, keep the plate inverted and tap firmly on a dry paper towel to remove as much Wash Buffer as possible.

Important: If samples were incubated overnight, increase the number of wash cycles to 8.

5. Add **100 μ L** of ***Phytophthora* Enzyme Conjugate** to each well.
6. Thoroughly mix the contents of the wells, as in step 2. Cover the wells with new tape or Parafilm and **incubate** for **1 hour** at **ambient temperature** on an **orbital plate shaker as described above**. Note: Shaking during incubation steps is mandatory where called for. Failure to do so will result in up to 50% loss in assay sensitivity.
7. Wash the wells again as described in step 4. Alternatively, perform four washes (300 μ L/well) with a microtiter plate or strip washer.
8. Add **100 μ L** of **Substrate** to each well. Mix thoroughly as in step 2. Cover the wells with new tape or Parafilm and incubate for **30 minutes** (for best results) at **ambient temperature**.
9. Add **100 μ L** of **Stop Solution** to each well and mix briefly. This will change the blue color in the wells to yellow. Read the plate at **450 nm**, with a reference wavelength between 600 and 650 nm. Read the stopped plate within 30 minutes; color may fade beyond that time.

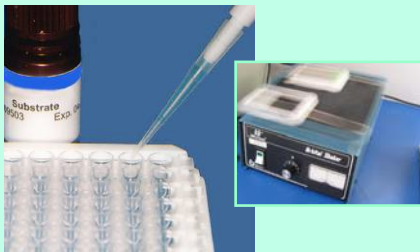
NOTE: Stop Solution is 1 N HCl. Handle carefully.



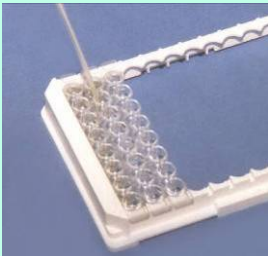
Strip Plate Wash option



Add Conjugate, mix, incubate, wash



Add substrate, mix, incubate



Add Stop Solution



Read plates in a Plate Reader
at 450 nm
within 30 minutes of the
addition of Stop Solution.

How to Interpret the Results

Spectrophotometric Measurement

Set the wavelength of the microtiter plate reader to **450 nanometers (nm)**. (If it has dual wavelength capability, use 600, 630 or 650 nm as the reference wavelength.)

Interpreting Results

Compare the Optical Density (OD) of the sample extracts to those of the mean Extraction Buffer wells, or preferably, to known-negative leaf extract wells, to determine presence or absence of *Phytophthora* in the sample extract. Samples with absorbances significantly greater than those of the Extraction Buffer and/or negative extract wells are presumed to be positive for *Phytophthora*.

General Guidelines:

- Mean OD of Extraction Buffer wells should not exceed 0.10.
- Mean OD of phytophthora-free leaf extracts should not exceed 0.15.

If your test results consistently fall outside these guidelines, please contact EnviroLogix' Technical Service.

Cross Reactivity

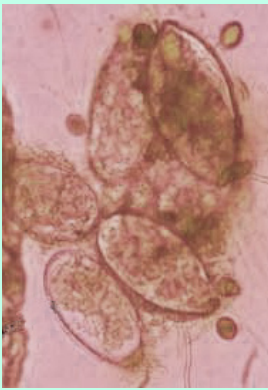
The antibody used to produce this kit was found to be reactive with the following *Phytophthora* species: *cactorum*, *cambivora*, *capsici*, *cinnamomi*, *citricola*, *citrophthora*, *cryptogea*, *cubayensis*, *drechsleri*, *erythroseptica*, *foliorum*, *fragariae*, *heveae*, *hibernalis*, *infestans*, *kernoviae*, *lateralis*, *macrochlamydiospora*, *megasperma*, *nicotianae*, *palmivora*, *ramorum*, *sojae*, *syringae*.

The antibody used to produce this kit was found to be not reactive with the following *Pythium* species: *catenulatum*, *graminicola*, *heterothallicum*, *intermedium*, *irregulare*, *myriotylum*, *pareocandrum*, *spinosum*, *sylvaticum*, *tolurosum*, *ultimum ultimum variant*, *vanterpoolii*. It is weakly reactive with *Pythium oligandrum* and strongly reactive with *Pythium aphanidermatum* and *Pythium vexans*. It has also been found to be not reactive with *Aspergillus*, *Rhizoctonium*, *Rhizopus*, and *Sclerotinia* spp.

Precautions and Notes

- Observe any applicable regulations, federal or state guidelines, or in-house lab safety protocols when disposing of samples and kit reagents.
- Store all QualiPlate components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not expose QualiPlate components to temperatures greater than 37°C (99°F) or less than 2°C (36°F).
- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before use.
- Do not use kit components after the expiration date.
- Do not use reagents or test plates from one QualiPlate with reagents or test plates from a different QualiPlate.
- **Do not expose Substrate to sunlight** during pipetting or while incubating in the test wells.

- Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
- Quality of results is dependent upon following the assay protocol as directed.
- Stopped plates must be read at a wavelength of **450 nanometers**—reading at 405 or 490 nm will result in incorrect interpretation of results.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.





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